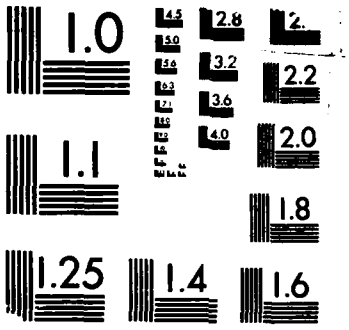
AD-A193 674 DEVELOPMENT OF VACCINES TO PREVENT WOUND INFECTIONS DUE TO ANAEROBIC BACTERIA(U) BRIGHAM AND WOMEN'S HOSPITAL BOSTON MA D L KASPER 02 APR 88 DAMD17-83-C-3239 1/1

UNCLASSIFIED F/G 6/15 NL



DTIC FILE COPY

(4)

AD-A193 674

REPORT DOCUMENTATION PAGE

DTIC ELECTE MAY 06 1988 S D

1b. RESTRICTIVE MARKINGS

2a. SECURITY CLASSIFICATION AUTHORITY

3. DISTRIBUTION/AVAILABILITY OF REPORT
Approved for public release; distribution unlimited

2b. DECLASSIFICATION/DOWNGRADING SCHEDULE

4. PERFORMING ORGANIZATION REPORT NUMBER(S)

5. MONITORING ORGANIZATION REPORT NUMBER(S)

6a. NAME OF PERFORMING ORGANIZATION
Brigham and Women's Hospital

6b. OFFICE SYMBOL (If applicable)

7a. NAME OF MONITORING ORGANIZATION

6c. ADDRESS (City, State, and ZIP Code)
Channing Laboratory, 180 Longwood Avenue
Boston, Massachusetts 02115

7b. ADDRESS (City, State, and ZIP Code)

8a. NAME OF FUNDING/SPONSORING ORGANIZATION
U.S. Army Medical Research & Development Command

8b. OFFICE SYMBOL (If applicable)

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
DAMD17-83-C-3239

8c. ADDRESS (City, State, and ZIP Code)
Fort Detrick
Frederick, Maryland 21701-5012

10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
62772A	3S1-62772874A	AA	266

11. TITLE (Include Security Classification)
DEVELOPMENT OF VACCINES TO PREVENT WOUND INFECTIONS DUE TO ANAEROBIC BACTERIA

12. PERSONAL AUTHOR(S)
Kasper, Dennis L.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 10/1/86 TO 5/15/87	April 2, 1988	

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
06	01		Human Hybrido Lymphokines; Anaerobic organisms; Lab animals; Volunteers
06	05		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Progress on the two major objectives of this contract were made during this year. One lot of capsular polysaccharide of Bacteroides fragilis vaccine was tested for protective capacity in mice and found to function to induce a protective response. One human volunteer was immunized without adverse reactions occurring and the immunogenicity will be assessed. On the second specific aim, major progress was made in producing a specific T cell factor by cloning lymphocytes in vitro to produce the protective lymphokine. A small molecular weight antigen specific factor has been produced from hybridoma clones. This factor is of T cell origin and protects mice against experimental infection with Bacteroides fragilis.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT
☐ UNCLASSIFIED/UNLIMITED ☑ SAME AS RPT. ☐ DTIC USERS

21. ABSTRACT SECURITY CLASSIFICATION
UNCLASSIFIED

22a. NAME OF RESPONSIBLE INDIVIDUAL
Mary Frances Bostian

22b. TELEPHONE (Include Area Code)
301/663-7325

22c. OFFICE SYMBOL
SGRD-RMI-S

DD FORM 1473, 84 MAR

83 APR edition may be used until exhausted.
All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

AD_____

DEVELOPMENT OF VACCINES TO PREVENT WOUND INFECTIOUS DUE TO ANAEROBIC BACTERIA

ANNUAL REPORT

Dennis L. Kasper, M.D.

August 15, 1987

U.S. ARMY MEDICAL RESEARCH DEVELOPMENT COMMAND
Fort Detrick
Frederick, Maryland 21701-5012

Contract DAMD-17-83-C-3239

Brigham and Women's Hospital
Boston, MA 02115

Progress Report

Specific aim I - Phase I trial of capsular polysaccharide vaccine assessing safety and immunogenicity.

A) Human vaccine trial

A single lot of Bacteroides fragilis capsular polysaccharide vaccine has been prepared from ATCC strain 23745. The organisms were cultured in a 14 liter fermentor in pre-reduced broth. Organisms were separated by centrifugation and the polysaccharide extracted with hot phenol-water. After removal of phenol and exhaustive dialysis of the water phase, the capsular polysaccharide was separated from the lipopolysaccharide by column chromatography in detergent containing buffer. The detergent was removed by alcohol solubilization and the precipitated polysaccharides treated with nuclease and protease to remove contaminants. After repeat chromatography to remove enzymes and digested materials, the final product was dialyzed exhaustively and lyophilized.(1) The vaccine eluted in the void volume of a Sepharose CL-4B column indicating that the size is large and probably aggregated. The vaccine lot (BF/-1R)was bottled at the Massachusetts Public Health Biology Laboratories. The safety and toxicity tests required by the Food and Drug Administration had been completed satisfactorily.

The bottled vaccine was shown to contain 1:9100 thimerosal which is used as a preservative. Sterility testing was performed and the lot was sterile; no growth was observed. Three rabbits were injected intravenously with 300 ug of polysaccharide (human dose 50 ug). The rabbits did not develop any fever to this injection over 4 hours observation. Two guinea pigs were injected with 500 ug of polysaccharide with normal growth and development continuing.

The final bottled product was checked for chemical composition (except for uronic acids) by comparison to the antigen lot prior to bottling. No significant chemical differences were noted. As a final test, the bottled material was checked for immunogenicity and protective capacity by immunization of C57/BL6 mice from Jackson Laboratories with a dose of 10 ug subcutaneously 3 times a week for three weeks. The immunization dose of 10 ug was delivered in 0.1 cc subcutaneously to each animal. A cohort group of littermate animals housed in the same room were used as a naive control. Eight animals were immunized with the capsular vaccine, and four animals were used as naive controls. Animals in both groups were challenged with 1 x 10^6 B. fragilis 23745 plus sterile cecal contents according to our previously published protocol (1).

The animals were sacrificed for evaluation of abscess formation. (2,3) Of the eight animals which were immunized, only one animal exhibited an abscess which revealed polymorphonuclear cells on gram stain. All four naive recipients of B. fragilis demonstrated large abscesses within the abdominal cavity. These data are consistent with our previously published findings for the capsular polysaccharide.

The above information was transmitted to the FDA, after waiting for questions or required clarification without receiving a response, we were assured that we could proceed with a phase I trial. Prior to beginning a human trial with the bottled polysaccharide, the vaccine was determined to be free of contaminating blood substances of the ABO group. A hemagglutination inhibition assay was performed and the vaccine did not inhibit hemagglutination whereas the endpoint of inhibition of the control was 1:512. Therefore the vaccine did not contain

A-1

contaminating blood group substances.

The first volunteer was given 50 ug of vaccine subcutaneously after obtaining preimmunization serum. The subject developed an immediate local hematoma but no subsequent soreness, induration,or fever. One week after vaccination 60 ml of blood was obtained in order to look for B cell activation in vitro as well as T cell stimulation. The B cells were cultivated in vitro and the supernatants were frozen for subsequent antibody determination. T cell proliferation studies on this one individual were negative. Nine further volunteers were immunized with capsular polysaccharide 50 ug SQ. No local or systemic reactions were observed.

The preliminary results on the immune response to this vaccine are now becoming available. The remaining six months of our contract will be devoted to evaluation of the immune response in these individuals.The preimmunization and 1 week post immunization sera have been tested in a hemagglutination assay using sheep red blood cells which were sensitized with the vaccine antigen. Six of ten volunteers had significant antibody responses (fourfold or greater rise) at this early date, suggesting a very successful immunization. The titers are shown in the table.

Table 1

Hemagglutination Titer

Volunteer	preimmunization	1 week post immunization
1	<1:1	1:16
2	1:4	1:8
3	1:1	1:2
4	1:8	1:32
5	1:32	>1:128
6	1:8	>1:32
7	1:2	>1:32
8	1:4	1:8
9	<1:1	1:1
10	1:64	1:64

Based on experience with other polysaccharide antigens in humans, we believe that it is likely that most of the non-responders will show a significant rise in 2 or 4 week post immunization sera. In fact two of four non-responders did have a twofold rise observed at one week.

B) Characterization of B. fragilis capsular polysaccharide variants

In the course of characterization of this antigen by biochemical means we discovered that the bacterial strain we have used for the past 13 years had undergone a significant antigenic shift in the structure of the capsular polysaccharide. Careful review of our laboratory records allowed us to isolate 3 variations of our original strain and we proceeded to further characterize these isolates. Characterization of these three isolates showed that they had

essentially identical lipopolysaccharides and similar outer membrane protein patterns on SDS-PAGE. The major biochemical difference in the capsular polysaccharides of the three isolates was in the quantitative galacturonic acid content. Many of the specific biochemical differences are included in this report.

Strain 23745 is the prototype Bacteroides fragilis strain of the American Type Culture Collection. We originally obtained this strain in 1973. We reported previously that in vitro passage of this strain resulted in partial loss of capsular polysaccharide and increased amounts of glycogen recoverable from outer membranes of this organism. This problem was avoided by in vivo passage of these organisms either through IV injection and recovery from mouse spleens or by using our abscess model for serial in vivo passage with recovery of organisms from abscess contents. Either of these methods resulted in a strain with a constant amount of recoverable capsule reasonably free of glycogen.

All lots of polysaccharide purified through 1982 contained ≈ 20-30% galacturonic acid when assayed by gas liquid chromatography following carbodiimide reduction and analyzing alditol acetate derivatives of the sugars, compared to non-reduced alidot acetate derivatives (4). Because preparation of carbodiimide reduced sugars is very time consuming, we had not checked every lot of polysaccharide for uronic acid content. Rather we prepared alditol acetate derivatives assessed for all other sugars, and assumed biochemical identity of lots based on the presence and quantity of all other sugars in this complex carbohydrate. However, to report the complete chemical composition of the vaccine lot to the FDA, we performed carbodiimide reduction. To our surprise this failed to demonstrate the presence of galacturonic acid. This surprising observation caused us to go back and check 14 previous lots prepared since 1982 for galacturonic acid content. All 14 lots could be traced back to three different passages of strain 23745, which we called 23745 var 61R1-1, 23745 var 61RC5-1 and 23745 var ENC-153. All three variants contained differing amounts of galacturonic acid, but had otherwise reasonably similar neutral amino or di-deoxyamino sugar contents (rhamnose, fucose, galactose, glucose, glucosamine, rhamnosamine, quinivosamine and fucosamine).

Table II

strain 23745	galacturonic acid content (%)
var 61R1-1	18%
var 61RC5-1	4%
var ENC-153	<2%

We felt that it was important to determine whether these capsular variations were isolated changes or whether other important outer membrane antigens had undergone antigenic drift as well.

Outer membranes were prepared from the three variants and analyzed for membrane proteins by SDS-PAGE (Fig. 1). All three variants had identical OM protein profiles except for var 61RC-5 which had an extra band at ≈27,000 MW. This band was of low density by Comassie Blue staining. Lipopolysaccharides were prepared from all three variants and analyzed for sugar and lipid content. The

carbohydrate analysis showed all three variants contained similar quantities of galactose, rhamnose and glucosamine and had the same 5 major fatty acids in similar abundance. These data suggest that the variation which has taken place in strain 23745 is limited and primarily isolated to the galacturonic acid portion of the capsular polysaccharide.

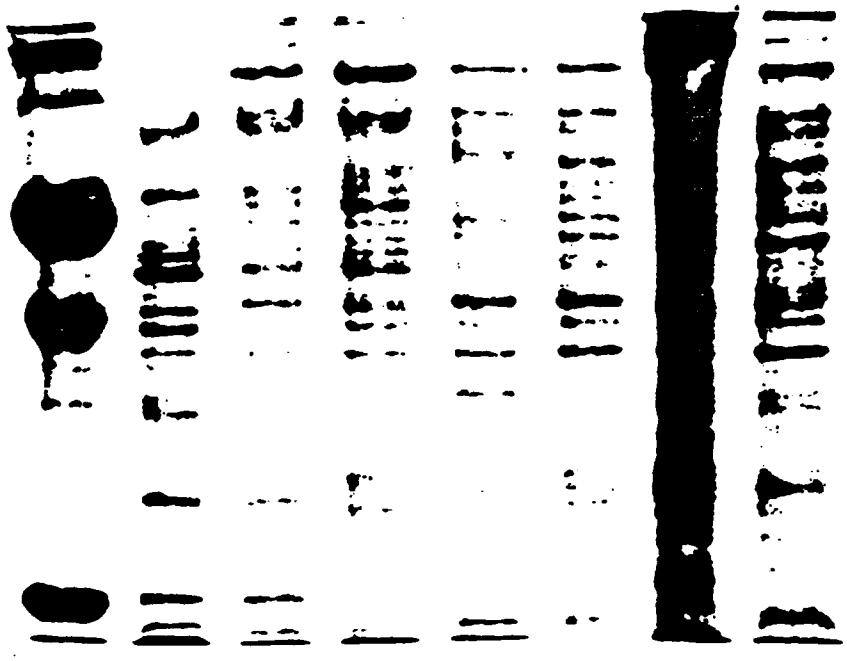
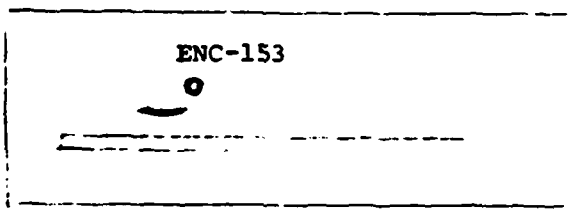


Figure 1

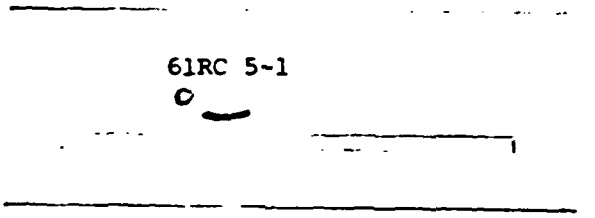
The vaccine lot served to complicate further the already complex situation. This lot contained no galacturonic acid and most peculiarly did not react in immunodiffusion with the 23745 rabbit antiserum. This polysaccharide lot however did have similar, but not completely identical quantitative conversion to polysaccharide made from var ENC-153. The vaccine preparation was made from var ENC-153, but from a different passage than that used for other lots. What encouraged us to proceed with the human trials was that this lot was protective in our abscess model in mice, and we had other data suggesting that the immunodeterminant site responsible for the T cell dependent protection was distinct from a site responsible for both humoral immunity and serological reactivity in immunodiffusion (not published). These data had suggested that the serologic site was galacturonic acid dependent. Therefore, it did not surprise us that the vaccine preparation lacking galacturonic acid still promoted T cell dependent immunity. Subsequent immunologic studies of the 3 variants mentioned above (not including the vaccine variant) showed the determinants were even more complex.

All three variants produced polysaccharides which reacted in immunodiffusion. Variants ENC-153 and 61RC5-1 gave single lines of identity with 23745 antiserum raised to capsular polysaccharides. Var 61R1-1 polysaccharide reacted identically with the other two variants, but two additional precipitin lines were observed. Immunoelectrophoresis of these three variant polysaccharide antigens also showed differences (Fig. 2) consistent with the net galacturonic acid content (net charge). The least mobile antigen was ENC-153 (least charged) and 61R1-1 was highly mobile. This raised the question of the purity of 61R1-1 polysaccharide which had 3 distinct lines visible on IEP and immunodiffusion. To determine whether contamination accounted for these multiple bands, HPLC analysis of var 61R1-1 polysaccharide was performed on an anion exchange column. Three distinct peaks were resolved from the original polysaccharide preparation. All three peaks contained identical neutral, amino and dideoxyamino sugar contents. This information suggests that the differing mobility of 61R1-1 on IEP reflects differing galacturonic acid content rather than contamination. Preparatory analysis currently is in progress to confirm this hypothesis.

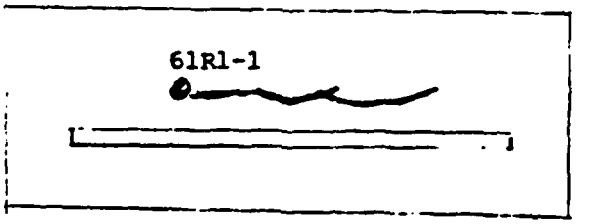
Figure 2



var ENC-153 capsular polysaccharide was electrophoresed from well and antiserum to strain 23745 was placed in trough



var 61RC 5-1 capsular polysaccharide and same antiserum



var 61R1-1 capsular polysaccharide and same antiserum

The relative ability of each of these variant polysaccharides to induce T cell dependent immunity in the mouse model of abscesses was tested. In addition, the ability to protect against abscesses caused by one or another of these variant 23745 bacterial strains was examined. Groups of 8-10 mice were immunized with 10 ug of one of the variant polysaccharides three times per week for 3 weeks. One week after the last injection, animals were challenged with the homologous variant as well as the other two variant organisms and protection against abscesses evaluated. All three variant strains reliably produced abscesses in naive mice. Results are shown in Table III.

Table III

Immunization with capsular polysaccharides of variant isolates from strain 23745 and cross challenges

Polysaccharide Immunization	Challenge Strain 61R1-1	61RC5-1	ENC 153
61RC5-1 10 ug	1/6	1/7	4/6
5 ug	-	5/8	-
1 ug	-	8/8	-
61R1-1 10 ug	2/10	2/9	9/9
5 ug	6/10	-	-
1 ug	8/10	-	-
ENC 153 10 ug	8/10	8/8	10/10
5 ug	-	-	10/10
1 ug	-	-	10/10

These results suggest that variant ENC-153 lacks a critical determinant necessary to induce T cell immunity. It should be pointed out that although the vaccine lot and the ENC-153 polysaccharide were both derived from the ENC-153 variant bacteria that there were immunologic differences between these two polysaccharides *in vitro* and *in vivo*. The ENC-153 variant reacted in immunodiffusion but failed to protect against abscesses in mice, while the vaccine polysaccharide protected mice but did not react in immunodiffusion.

To help clarify the serologic relatedness or lack thereof, of var 61R1-1 and the vaccine lot capsular polysaccharides, rabbits were immunized with these antigens. Post immunization sera were tested in the hemagglutination assay using sheep red blood cells sensitized with each antigen. Results appear in Table IV.

Table IV

Rabbit Antibody Response to
B. fragilis capsular polysaccharide HA titer

capsular polysaccharide used for immunization	RBC sensitized with 61R1-1 capsule		RBC sensitized with lot 1 vaccine	
	pre	post	pre	post
vaccine lot	<1:1	1:2	<1:1	>1:32
61R1-1	<1:1	>1:16	<1:1	1:2

These data show that rabbits respond primarily to the immunizing antigen with little heterologous response.

Specific Aim II - *In vitro* production of specific T cell factor by cloning of lymphocytes in tissue culture and hybridoma technology.

In order to produce a T cell hybridoma making specific T cell factor we have fused cloned splenic T cells from mice immunized with *B. fragilis* CP with a thymoma parent line (BW), thereby immortalizing the cells. Nylon-wool column passed immune spleen cells were cultivated *in vitro* with Con A supernatant (IL-2), capsular polysaccharide and irradiated immune spleen cells to serve as stimulator cells. The growth factor dependent cell lines were cloned by limiting dilution. The putative clones then were recloned by the same methods. Cloned cells were mixed with BW 5147 thymoma cells at a ratio of 5:1. After centrifugation, a solution of polyethylene glycol (PEG) AND 15% dimethylsulfoxide
(DMSO) in Dulbecco's Minimal Essential Medium (DMEM) without serum was added to the pellet. Cells were centrifuged and resuspended in DMEM-HAT medium with 20% fetal calf serum (FCS). Cells were aliquoted at 3×10^3 in wells of a microtiter plate and fed 10^5 syngeneic thymocytes to improve viability.

Hybrids were fed HAT medium for one month to eliminate residual tumor parent cells and then switched to DMEM with 10% FCS. Fusion of a T cell line was ensured by sorting for Thy 1.2 cells on a fluorescence activated cell sorter and by selecting cells which tolerate HAT medium since BW thymoma cells are Thy 1.1 and sensitive to HAT medium. Supernatants from several fusions and from the BW parent line harvested without antibiotics in the medium were screened for ITF activity in the modified bactericidal assay. A supernatant referred to below as 2E8 combined with C' killed B. fragilis in vitro while the parent BW and another supernatant, 2D3, failed to demonstrate in vitro killing. These hybridoma supernatants (Table V) were tested for protective activity in mice. 2E8 protected mice, while BW and 2D3 were not protective.

Table V

Activity of Hybridoma Supernatants in Protection against Abscesses

Lysate or supernatant transferred	# of mice with abscesses/total
Naive T cell factor	9/10 (10%)*
Immune T cell factor	2/10 (80%)
BW supernatant	12/13 (7.7%)
2D3 supernatant	10/14 (28.6%)
2E8 supernatant	2/15 (86.7%)

* ( ) = % protection

To assess whether 2E8 was dialyzable, 3cc of 2E8 supernatant were placed in a dialysis membrane and dialyzed against 24 cc of 5mM ammonium acetate pH 7.1 for 24 h a 4^{o} C with two changes of the dialysate. 0.2 cc of pooled dialysate was protective, while the dialysis bag contents lost protective capacity against experimental abscess formation (Table VI).

Table VI

Activity of Dialyzed Hybridoma Supernatants in Protection against Abscesses

Supernatant transferred	# of mice with abscesses/total
BW supernatant	4/4 (0%)*
2E8 supernatant	0/5 (100%)
2E8 supernatant dialysis bag contents	7/7 (0%)
2E8 supernatant dialysate	0/8 (100%)

* ( ) = % protection

Specificity of the hybridoma-produced factor was assessed by incubation of 2E8 supernatant with sheep red blood cells coupled to B. fragilis CP, type III group B Streptococcus CP, or SRBC alone. Only incubation with SRBC linked TO B. fragilis CP removed protective activity from the 2E8 supernatant (Table VII).

Table VII

Supernatants	Treatment of supernatants	# of mice with abscesses/total
2E8	absorbed with SRBC	0/8 (100%)
2E8	absorbed with SRBC coupled to GBS polysaccharide	0/8 (100%)
2E8	absorbed with SRBC coupled to B. fragilis capsular polysaccharide	8/8 (0%)

* ( ) = % protection

Therefore, we demonstrated that one of our hybridomas produced a small molecular weight, antigen-specific factor capable of protecting mice against intraabdominal abscesses caused by B. fragilis. The properties of this hybridoma supernatant were identical to the immune T cell factor (ITF) prepared by lysing splenic T cells from immunized mice. We, therefore, have accomplished our goal of producing active T cell factor in large quantities and can pursue further characterization.

Xenogenic transfer of ITF

We had proposed giving mouse hybridoma factors to rats to determine xenogenic protection. We have completed a preliminary study using hybridoma 2E8 and parent BW supernatant as a control. In this study Wistar Lewis Rats were given 0.3cc of either 2E8 or BW supernatants by the intracardiac route. Twenty four hours later, gelatin capsules containing 5×10^6 B. fragilis, sterile cecal contents and Barium sulfate were implanted intraperitoneally. One week later the animals were necropsied and assessed for abscesses. Seven of seven rats receiving BW supernatant developed i.p. abscesses compared to 1/7 rats receiving 2E8. These results suggest xenogenic protection by the immune factor produced by hybridoma 2E8.

REFERENCES

1.Kasper, DL, Weintraub A, Lindberg AA, Lonngren J. Capsular polysaccharides and lipopolysaccharides from two B. fragilis reference strains: Chemical and immunochemical characterization. J Bacteriol 1983; 153:991-7.

2.Shapiro ME, Onderdonk AB, Kasper DL, Finberg RW. Cellular immunity to Bacteroides fragilis capsular polysaccharide. J Exp Med 1982; 154:1188-97.

3.Kasper DL, Lindberg AA, Weintraub A, Onderdonk AB, Lonngren J. Capsular polysaccharides and lipopolysaccharides from two strains of Bacteroides fragilis. Rev Infect Dis 1984; 6:S25-S29.

4.Onderdonk AB, Shapiro ME, Finberg RW, Zaleznik DF, Kasper DL. Use of model of intraabdominal sepsis for studies of the pathogenicity of Bacteroides fragilis. Rev Infect Dis 1984; 6:S91-S95.

END
DATE
FILMED
7-88
DTIC